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Instruments and techniques

Extra- and intracellular hydrogen ion-selective microelectrode based on neutral carriers with extended pH response range in acid media

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Abstract. A series of new neutral hydrogen ion carriers suitable for application in H^+-selective microelectrodes is presented. One carrier (ETH 1907) proves to be superior to tridodecylamine currently very much in use. Microelectrodes based on ETH 1907 in an optimized membrane composition exhibit a linear dynamic response function from pH 2 to 9 extended into the acidic range, a response time ≤ 5 s, and a resistance of about 35 GΩ for a tip diameter of about 1 μm. This makes the electrode suitable for measurements at normal physiological intracellular pH as well as in acid physiological media. Measurements using this microelectrode in proximal tubule cells of isolated perfused frog kidney are presented.

Key words: Neutral carriers – Microelectrode – H^+ selectivity – Proximal tubule – Frog kidney

Introduction

Most cells maintain their intracellular pH between 7.0 and 7.4, which is about one pH unit higher than would be expected if hydrogen ions were passively distributed across the plasma membrane. In vertebrate cells a close regulation of cytosolic pH is achieved by extruding hydrogen ions through the presence of a Na^+/H^+ exchanger driven by the steep Na^+ gradient. The inflowing sodium ions are removed by the Na^+/K^+ ATPase. Deviations from this strictly controlled intracellular normal pH range may however occur. Relatively low pH values have been assessed under various conditions: in the stomach of fish [pH_o (stomach fluid) ≥ 3 [9]), for severe acidosis during complete brain ischemia (pH_o (interstitial) ≥ 5.3 [6]), in old rhizoid cells (pH_i 4.8 [4]), in vacuoles (pH(vacuolar) ~ 4.5 [3], pH(vacuolar) ~ 6 [7]), and after acid loading with ammonium ions (e.g. pH_i 5.7 [11], pH_i 6.5 [14]).

Glass membrane microelectrodes exhibit extremely high H^+ selectivities and cover a wide dynamic pH range with Nernstian response. During the last five years a liquid membrane microelectrode based on the hydrogen ion carrier tridodecylamine (TDDA) [2] has replaced the glass microelectrodes in electrophysiology to a large extent. Liquid membrane microelectrodes are much easier to prepare and allow the use of small tip diameters and/or double-barrelled micropipette configurations. The dynamic pH range of TDDA-based microelectrodes is found to be between pH ~ 5.5 and pH 12 [2, 5] which obviously limits their use in acid media. Furthermore, very fine tip microelectrodes (< 0.5 μm) for use in very small cells display sometimes chloride sensitivity at pH values ≤ 7.0. Recently, it was shown that the dynamic pH range of neutral carrier-based liquid membrane electrodes is directly related to the acidity constant of the active site of the ionophore [12]. Here we report on a hydrogen ion-selective microelectrode containing a new H^+ ionophore with a dynamic pH range from 2 to 9 in physiological electrolyte solutions.

Methods

Electrode system. Cells of the following type were used:

Ag; AgCl, 3 M KCl | sample solution ‖

microreference electrode

membrane ‖ buffer solution pH 7

(0.04 M KH_2PO_4, 0.023 M NaOH, 0.015 M NaCl), AgCl; Ag

ion-selective microelectrode

Ion-selective microelectrodes. Glass micropipettes were drawn from clean single-barrelled borosilicate glass capillaries without internal filament (Clark Electrochemical Instruments, GC 150T-15). Under the microscope the tips of the micropipettes were broken to about 1 μm on a polished plexiglass rod. Several broken micropipettes were examined by scanning electron microscopy, the observed mean tip diameters being 0.95 ± 0.08 μm ($n = 7$) [1]. The micropipettes were placed in an oven, covered with a glass beaker, and predried at 150° C for 30 min. After increasing the temperature to 200° C, about 50 μl of N-(trimethylsilyl)dimethylamine (purum; Fluka AG, Buchs, Switzerland) were injected into the beaker and the reagent was allowed to react with the glass surface in the vapour phase at 200° C for 30 min. In a dust-free environment the silanized micropipettes could be stored over silica gel for several days.

The ion-selective liquid of the optimized H^+ microelectrode consisted of 6 wt.-% of the H^+-selective carrier ETH 1907 and 1 wt.-% of potassium tetrakis(4-chlorophenyl)borate (KTpClPB; purum p.a., Fluka) in o-nitrophenyl octyl ether (o-NPOE; p.a. for ion-selective elec-

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trodes, Fluka). After injecting the internal filling solution through the stem of the micropipettes (backfilling), they were immersed into the membrane solution for 10 s. The resulting filling height was between 100 µm and 300 µm.

For the reference electrodes the tips of nonsilanized micropipettes were broken to 2–3 µm and filled with 3 M KCl solution. In some cases, the microreference electrodes could be used for several weeks.

EMF measurements. The EMF measurements were performed at 20°C using FET operational amplifiers AD 515L (for details of the equipment see [2]). Membrane resistances were measured using the method of potential reduction by known shunt.

Syntheses. The hydrogen ion carrier tridodecylamine (TDDA) is commercially available (Fluka). The synthesis of the carrier 1-octadecanoyl-4-(2-pyridyl)piperazine (ETH 2003) has been described earlier [12].

The ionophore 4-nonadecylpyridine (ETH 1907) was prepared according to the procedure of Osuch and Levine [13]. The crude product was purified by flash chromatography (silica gel, hexane/ethyl acetate 7:3) and recrystallization from pentane (melting point: 38.5–39°C; 35% yield). Elemental analysis calculated for $C_{24}H_{43}N$: C 83.41%, H 12.54%, N 4.05%; found C 83.72%, H 12.76%, N 4.00%.

The ionophore N-octadecylimidazole (ETH 2111) was obtained from imidazole (3 g, 44.1 mmol; Merck, Darmstadt, FRG) which was dissolved in 100 ml methanol (Fluka) and first treated with 10 ml aqueous 10 M NaOH and then with 1-bromooctadecane (23.5 g, 70.5 mmol; Fluka, purum). The mixture was refluxed at 80°C for 4 h. The water/methanol mixture was removed and the residue dissolved in methylene chloride and extracted 5 times with water. The organic phase was dried over $MgSO_4$ and the solvent evaporated (crude product: 13.9 g; 98% yield). The product was purified by flash chromatography (silica gel 60, Fluka) with ethyl acetate and recrystallized from pentane (3.0 g; 21% yield). Elemental analysis calculated for $C_{21}H_{40}N_2$: C 78.68%, H 12.58%, N 8.74%. Found: C 78.77%, H 12.65%, N 8.71%.

Results and discussion

When characterizing new neutral carriers for hydrogen ions the observation has repeatedly been made that the replacement of sodium tetraphenylborate (NaTPB) by KTpClPB as additive in the membrane phase of microelectrodes apparently does not alter the electromotive behaviour of the sensor, yet has several advantages. The incorporation of anionic sites into the membrane phase is necessary for a proper electromotive performance of the electrode as well as for reducing its electrical resistance. Due to its higher lipophilicity KTpClPB is less water-soluble and more soluble in the membrane solvent than NaTPB, thus helping to avoid some problems [2, 7] in the preparation of the membrane cocktail. KTpClPB readily dissolves in the often used membrane solvent o-NPOE; consequently, it was found that the treatment of the membrane cocktail with gaseous CO_2 is not necessary. The initial treatment of the TDDA-based cocktail with CO_2 is controversial, no convincing mechanistic reasons for its requirement having been given so far. Most likely it is related to the poor solubility of NaTPB in o-NPOE. Therefore, all further studies with TDDA as well as with the new carriers (Fig. 1) have been performed with KTpClPB as additive and without any CO_2 treatment of the membrane phase.

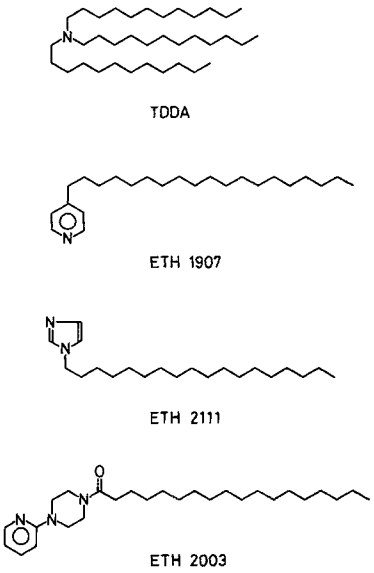

Fig. 1. Constitutions of neutral carriers for H^+-selective microelectrodes

Table 1 gives a comparison of properties of single-barrelled 1 µm-microelectrodes based on membrane cocktails containing four selected H^+ carriers (Fig. 1) dissolved in o-NPOE with KTpClPB as additive. The microelectrodes have comparable electrical membrane resistances and show pH response functions with slightly subnernstian slopes. As expected, the lower the pK value of the ionophore, the lower is the pH limit of the linear dynamic response range of the corresponding sensor [12]. Simultaneously, the detection limit in the alkaline region is shifted to lower pH values, which can be explained by an increased interference from sample cations. Indeed, microelectrodes based on the H^+ carrier with the lowest pK value (ETH 2003) may be applied to rather low pH values but their use in the physiological pH range is strongly limited. The pH response of microelectrodes with N-octadecylimidazole (ETH 2111) ideally covers the normal physiological pH range but the detection limit in acid media is shifted only slightly in comparison with TDDA-based microelectrodes (Table 1). Furthermore, the electromotive behaviour of sensors based on ETH 2111 is disturbed after contact with acid solutions ($pH \leq 4$). An almost ideal dynamic pH range is obtained with 4-nonadecylpyridine (ETH 1907): When incorporated into microelectrodes their dynamic pH response covers the physiological range and is extended by more than 3 pH units into the acidic region as compared to microelectrodes based on TDDA (Table 1).

In Fig. 2 the pH response functions of microelectrode cell assemblies with TDDA and ETH 1907 are compared. It is obvious that the latter exhibits a physiologically more relevant dynamic pH range than that based on TDDA. The reduced detection limit at high pH values indicates a poorer selectivity towards cations (see Table 1) but is still sufficient for studies in solutions with intracellular ion backgrounds (Fig. 3). During preliminary physiological studies it was observed that the lipophilic cation mepacrine interferes significantly even at low concentrations (10^{-6} M) with microelectrodes based on ETH 1907 but not with microelec-

Table 1. Properties of single-barrelled H^+-selective liquid membrane microelectrodes (tip diameter: 1 µm) at 20° C

	TDDA (10 wt.-%) KTpClPB (1 wt.-%) o-NPOE (89 wt.-%)	ETH 2111 (5 wt.-%) KTpClPB (1 wt.-%) o-NPOE (94 wt.-%)	ETH 1907 (6 wt.-%) KTpClPB (1 wt.-%) o-NPOE (93 wt.-%)	ETH 2003 (4 wt.-%) KTpClPB (1 wt.-%) o-NPOE (95 wt.-%)
pK[a]	10.6 [12]	7.7	6.1	5.6 [12]
Linear range[b] [pH]	>12–5.5	11–4	9–2	7–2
Slope [mV][c]	57.7±0.5	51.7±2.3	56.7±1.0	57.1±0.3
Selectivity coefficient K^{Pot}_{HK}	$<2 \cdot 10^{-11}$		$2 \cdot 10^{-9}$	
K^{Pot}_{HNa}			$2 \cdot 10^{-10}$	
Electrical membrane resistance[d] [GΩ]	35±13 (n = 7)	29±8 (n = 2)	35±9 (n = 8)	15±4 (n = 4)

[a] Acidity constant of the neutral carrier in water, determined on a water-soluble short chain homologue
[b] pH range of the electrode function over which the slope was determined; background: 0.1 M phosphate (potassium) buffer
[c] Average from n = 4 microelectrodes except for ETH 1907 where n = 6; standard deviations given
[d] Tip diameter: 1 µm; filling height: 100–300 µm. Standard deviations given

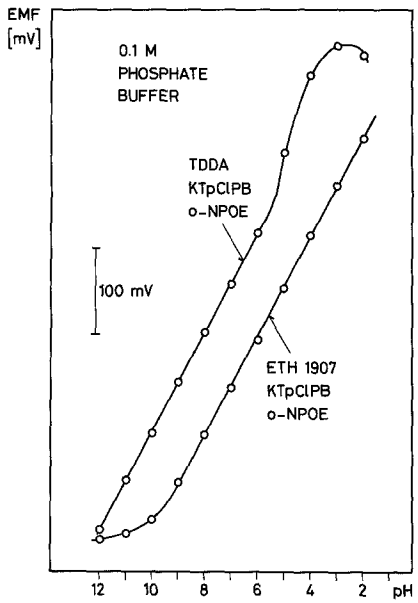

Fig. 2. EMF response of microelectrode cell assemblies based on the carriers TDDA and ETH 1907 to buffer solutions of different pH

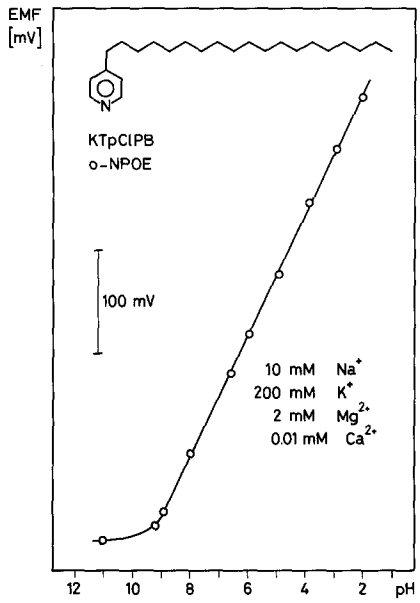

Fig. 3. EMF response of the microelectrode cell assembly based on 4-nonadecylpyridine (ETH 1907) to different H^+ activities at a representative intracellular ion background

trodes based on ETH 2111. Consequently, if such artifically added chemicals are involved, their possible interference on the pH electrode response function should be carefully evaluated.

Due to the relatively high electrical membrane resistance, which is typical for neutral carrier-based microelectrodes, the response time is in the range of seconds. The resistance of the TDDA-based microelectrodes is somewhat lower than originally reported [2]. This is not due to the new additive but can be explained by the much smaller filling height of the membrane solution in the microelectrodes used here. The irregularities in the EMF tracing by a step change of pH (Fig. 4) is most probably due to inadequate mixing of the added solution to perform this step change.

From measurements with four different microelectrodes mean drifts of 1.0 mV/h during the first 4 h and 0.2 mV/h within 62 h were found.

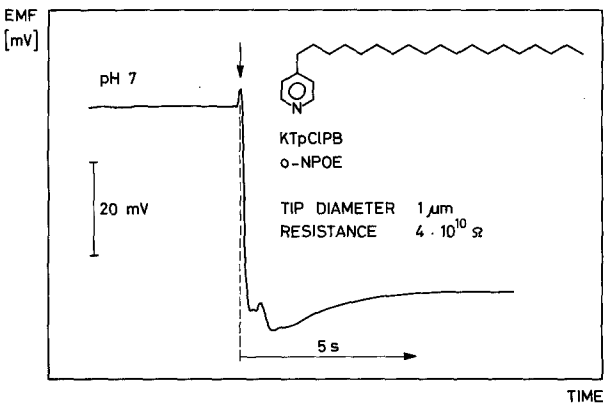

Fig. 4. Response of the microelectrode cell assembly based on ETH 1907 to a pH increase of the sample (phosphate buffer of pH 7) by an addition (*arrow*) of a slightly alkaline phosphate buffer solution

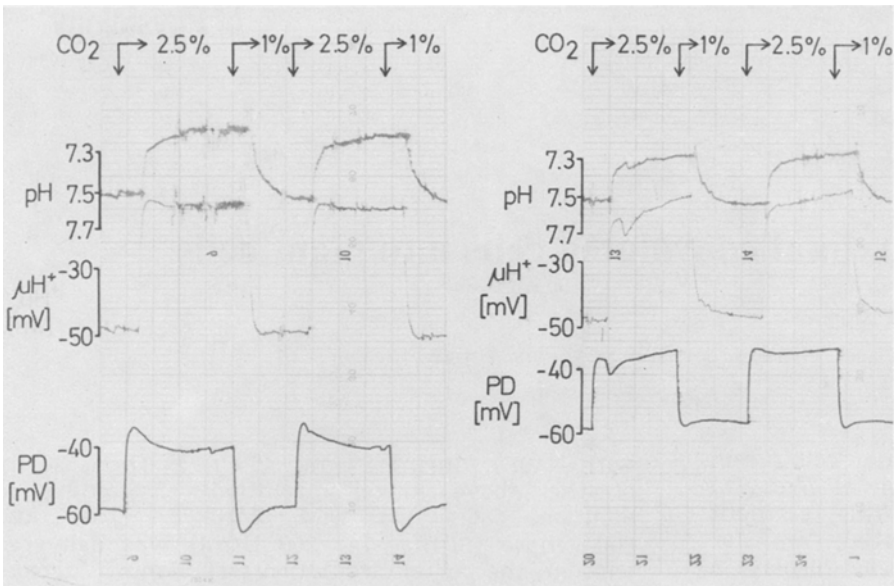

Fig. 5
Original tracing of intracellular pH determinations in proximal tubules of isolated perfused frog kidney. The potential difference across the peritubular cell membrane was recorded with a conventional (PD) and a pH-sensitive microelectrode (μH^+) based on ETH 1907 (*left tracing*) or TDDA (*right tracing*). The differential recording (μH^+-PD) reflects the intracellular pH. During the experiment peritubular CO_2 was increased from 1% (pH 7.7) to 2.5% (pH 7.3). Composition of the perfusate (mmol/l): 75 NaCl, 3.0 KCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 10 $NaHCO_3$, 0.6 Na_2HPO_4, 0.1 NaH_2PO_4, 10 glucose

Figure 5 displays the performance of microelectrodes (with tips < 0.5 μm) filled with either TDDA or ETH 1907 and impaled into a proximal tubule cell. For this experiment frog kidneys were isolated and perfused as described elsewhere [10]. During the experiment peritubular pCO_2 was increased. This manoeuvre was followed by rapid and reversible alterations in the cell membrane potential and intracellular pH. The changes in intracellular pH reflect the high permeability of the cell membranes to CO_2. The depolarization of the cell membrane is at least partly due to the pH sensitivity of the potassium conductance described previously [8]. In these experiments, the performance of the two microelectrodes is almost identical. Thus, microelectrodes filled with ETH 1907 are similarly suitable for intracellular impalements as the well established microelectrodes filled with TDDA. Given the better resolution at low pH and less susceptibility to chloride interference of very fine tip microelectrodes, ETH 1907 may prove even more useful for intracellular pH measurement.

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